

THE  
BOTANICAL GAZETTE

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CHEMICAL CONSTITUENTS OF AMARANTHUS  
RETROFLEXUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 254

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(WITH ELEVEN FIGURES)

**Introduction**

It is a well known fact that weeds retard the development of cultural plants. This is due to a number of causes: use of water, shading, use of nutrient salts, etc. It has been claimed for various species of *Amaranthus* that they not only absorb nitrates to care for their nutrient needs, but that they store much nitrogen as nitrate. If this be true, this genus has an excellent adaptation to enable it to combat cultural plants, for nitrate supply is a common limiting factor for crop growth. In order to investigate this statement, to locate the place of nitrate storage, and to determine the amount of nitrogen used otherwise by this plant, separate analyses were made of roots, stems, leaves, and branches of *Amaranthus retroflexus* at various stages and under various conditions of growth. The amount of the several carbohydrates was also determined in each analysis, in order to calculate the carbohydrate-nitrogen ratio which is lately receiving so much attention. A tissue analysis of the seeds was also made in an endeavor to ascertain more fully the chemical constituency of this plant, with the hope of learning more of the peculiar germinative behavior of these seeds.

### Historical

The first chemical analysis of *Amaranthus* was made by BOUTIN (1), a French chemist, in 1873. Housewives of that time were using these plants to clean their cooking utensils. This fact gave it some commercial importance and brought it to the attention of chemists. It was thought that the ability to "cut grease" must be due to acids in the plant. To verify this BOUTIN incinerated 100 gm. dry weight of the entire plant, and obtained 16 gm. residue. Water was added to leach out the soluble salts. The soluble portion weighed 8 gm. He called this potassium carbonate, and calculated the equivalent weight in grams of potassium nitrate, and found it to be 11.68, or 11.68 per cent. BOUTIN concluded that this plant was neutral in reaction on account of the presence of the neutral salt  $\text{KNO}_3$  (as a matter of fact this plant is acid in reaction). Later (2) he made analyses of the other species of *Amaranthus* by the same method, to determine the amount of  $\text{KNO}_3$ . The result of his analysis was as follows: *A. atropurpureus* contains 22.77 per cent  $\text{KNO}_3$  (one kilogram gives 31 gm. N and 103.5 gm. K); *A. Blitum* contains 11.68 per cent  $\text{KNO}_3$ ; *A. ruber* contains 16 per cent  $\text{KNO}_3$  (one kilogram gives 22 gm. N and 72 gm. K). It is evident that BOUTIN's method is not an accurate quantitative method of determining the amount of nitrate present. On the other hand, he demonstrated by other qualitative methods that the several species of *Amaranthus* studied contain a large amount of potassium nitrate. BROSSET (3) suggests the use of these plants as a fertilizer.

PAMMEL and DOX (15), in 1917, made microchemical tests of three common pigweeds, *A. blitoides*, *A. graecizans*, and *A. retroflexus*, and found them to contain abundant starch, some protein, and a little fat. In addition they made the Kjeldahl-Gunning nitrogen determination and found these species to have 1.88, 2.32, and 2.49 per cent of nitrogen. Multiplying by the factor 6.25, they obtained 11.75, 14.52, and 15.59 per cent of protein respectively.

HARDING and EGGE (8) made an analysis of the seeds of *A. retroflexus* for fats, protein, starch, sugars, hemicellulose, crude fiber, and tannin.

Of late much significance is being attached to the carbohydrate-nitrogen ratio of plant tissues, or, as FISCHER (6) puts it, the  $\frac{C}{N}$ . On the basis of work done by him and others, FISCHER makes the following generalizations. If the value of  $\frac{C}{N}$  rises by an increase in the amount of carbon, or by a decrease in the amount of nitrogen furnished the plant, there is an increase in the amount of flowering. If the value of  $\frac{C}{N}$  drops by a decrease in the amount of carbon, or by an increase in the amount of nitrogen furnished the plant, there is an increase of vegetative growth and a reduction of flowering. Briefly stated, a great preponderance of carbohydrates in plants favors flowering. Since the carbon of plants is fixed from the carbon dioxide of the air by photosynthesis, conditions that favor photosynthesis will tend to increase the ratio, and according to FISCHER the flower production. He found that increased partial pressures of carbon dioxide in the air had this effect. Since nitrogen is absorbed from the soil in the form of nitrates, conditions that favor nitrate absorption will decrease the ratio, and according to FISCHER favor vegetation.

KRAUS and KRAYBILL (12), on the basis of much more critical work, including numerous cultures, tissue analyses, and micro-chemical and anatomical studies, conclude that a very high  $\frac{\text{carbohydrate}}{N}$  value gives little vegetation and little or no reproduction; a medium  $\frac{\text{carbohydrate}}{N}$  value gives moderate vegetation and good reproduction; and a low  $\frac{\text{carbohydrate}}{N}$  value gives vigorous vegetation and little reproduction. Through their extreme conditions of culture, withholding nitrates, it is probable that KRAUS and KRAYBILL got much higher carbohydrate plants than FISCHER obtained in his cultures, hence their conclusion that very high  $\frac{\text{carbohydrate}}{N}$  gives little vegetation or reproduction. In short, FISCHER worked only on the portion of the  $\frac{C}{N}$  curve that induced fair vegetation and good reproduction or extreme vegetation and little reproduction, but not on the extreme of the curve that greatly

reduces both vegetation and reproduction. KRAUS and KRAYBILL cite literature showing that various conditions that greatly retard growth produce high carbohydrate plants. It seems that such conditions retard the use of carbohydrates for building new tissue to a greater degree than they do photosynthesis, and thereby lead to an accumulation of carbohydrates.

HEDLUND (9) finds that under like cultural conditions those varieties of winter wheat that have a higher percentage dry weight in the autumn are generally more winter hardy than the ones having a low percentage dry weight, and that cultural conditions that make for high percentage dry weight in any variety also make for winter hardiness. He finds, as do KRAUS and KRAYBILL, that high percentage dry weight is due to high percentage carbohydrate, and therefore high  $\frac{\text{carbohydrate}}{N}$ .

RIBERA (16) finds that all cultural conditions that increase the percentage dry weight in wheat decrease lodging. From this and the two investigations previously mentioned it is evident that high  $\frac{\text{carbohydrate}}{N}$  increases straw strength and decreases lodging.

High percentage of carbohydrate is said to increase hardiness, at least in part, by the greater amount of glucose present, and it may increase straw strength by inducing greater development of mechanical tissue along with greater thickness of walls, as KRAUS and KRAYBILL found for certain tissues of the tomato.

## Methods and results

### GREEN PLANT AT VARIOUS STAGES OF DEVELOPMENT

*Preparation of samples.*—Samples were secured on June 3, June 20, and July 8 consecutively from a vacant lot on 59th Street and Ingleside Avenue, Chicago. On June 20 samples were taken from two places, namely, the manure pile (rich soil) and the knoll (poor soil) for comparative work. The soil particles adhering to the roots and rootlets were removed by running water from a filter pump. As the velocity of water was very great, the soil particles were removed without difficulty. The roots were partially dried by the air current from the laboratory air line, and

finally dried by the use of paper towels. The roots, stems, and leaves were detached for separate analysis. The roots and the main stems were separated by cutting just between the cotyledon scar and the first branching rootlet. The leaf blades with the petioles were separated from the stem. Each portion was weighed and the length and diameter measured. Table I gives a brief description of the three consecutive samples.

TABLE I  
THE GREEN PLANT AT VARIOUS STAGES OF GROWTH

Measurement	June 3 collection	June 20 collection	July 8 collection
	Inches	Inches	Inches
Average height.....	1.00-4.00	6.00-8.00	20.00
Taproot {length.....	1.00-1.50	4.00-6.00	4.00- 6.00
{diameter.....	.....	0.10-1.00	1.00- 1.20
Secondary rootlets {length.....	None	0.10-1.00	8.00-14.00
{diameter..	None	.....	0.13- 0.25
Stems {length.....	1.00-4.00	6.00-8.00	20.00
{diameter.....	0.13-0.25	0.37-0.75	0.13- 0.25
Lateral branches {length.....	None	0.10-1.50	14.00
{diameter....	None	.....	0.25- 0.50
Seed head.....	None	None	0.25- 1.00
Green weight	Grams	Grams	Grams
Roots.....	26.00	28.50	29.20
Main stems.....	52.05	97.40	88.30
Branches.....	None	None	80.45
Leaves.....	188.90	142.20	110.25

The green samples were then immediately put in a freezing chamber, allowed to freeze overnight, and ground in a meat grinder the next morning. The freezing prevents losses. In the frozen condition no juices ooze out or spatter in the manipulation. The samples were boiled with 95 per cent alcohol to destroy the enzymes, and were then transferred to extraction cups, with filter paper thimbles, previously dried and weighed. The tissues were fractionated according to KOCH's (11) scheme for tissue analysis, namely, the lipin or ether soluble fraction ( $F_1$ ), the alcohol water soluble fraction ( $F_2$ ), and the insoluble fraction ( $F_3$ ). In the green plant  $F_2$  was comparatively small, consisting of chlorophyll and extracts of various pigments. The  $F_1$  was put together with  $F_2$  for the following carbohydrate and nitrogen estimation.

## ANALYSES

*Nitrogen compounds*

NITRATES.—The nitrates were determined by the Schlösing-Wagner method (14) as modified by KOCH for use in his laboratory. The modification consists essentially in the use of an inverted burette instead of a tube sealed at one end, and of the Van Slyke apparatus (only volumetric tube and Hemple pipette), to measure the true volume of nitric oxide. The principle of the method is:  $3\text{Fe}^{++} + \text{NO}_3^- + 4\text{H}^+ \rightarrow 3\text{Fe}^{+++} + \text{NO gas} + 2\text{H}_2\text{O}$ . Therefore 1 mol. NO gas gives an equivalent of 62 gm. of  $\text{NO}_3$ , or 1 cc. of gas =  $\frac{62}{22400} = 2.77$  mg.  $\text{NO}_3$ .

In order to determine the accuracy of this method, a known solution of  $\text{KNO}_3$  (0.5 per cent) was used. Four consecutive determinations with 10 cc. of the known solution were made. The average volume of nitric oxide gas for each 10 cc. solution, calculated to standard condition, was 11.12 cc. The theoretical volume for 10 cc. of 0.5 per cent  $\text{KNO}_3$  is 11.078 cc.

The determination of nitrates in the samples was made by taking an aliquot of the soluble fractions ( $\text{F}_1$  and  $\text{F}_2$ ). The nitric oxide gas driven over was caught in an inverted burette which had previously been filled with 40 per cent NaOH to absorb the  $\text{CO}_2$  and neutralize the hydrochloric acid (HCl gas will come over when the hydrochloric acid concentration reaches 20 per cent in the boiling flask). The burette containing the nitric oxide gas was set aside and allowed to cool to room temperature; then the nitric oxide gas was transferred to the Van Slyke apparatus. The total volume and the volume of unabsorbed gas were recorded. The absorbed volume by the alkaline  $\text{KMnO}_4$  in Hemple pipette is that of nitric oxide. This volume was then calculated to standard volume from temperature and barometric pressure, for example:

	I	II
Aliquot in cc. used.....	25.0	25.0
Total volume of gas (nitric oxide+air).....	4.80	4.85
Volume of unabsorbed gas (air).....	1.27	1.26
Volume of (absorbed) nitric oxide.....	3.53	3.59
Barometric pressure = 746.9; temperature 20.5° C.		
Volume at standard condition.....	3.23	3.27

	I	II
Equivalent in milligrams of $\text{NO}_3$ .....	8.95	9.06
Milligrams of dry substance (25 cc.) used.....	107.75	107.75
Percentage $\text{NO}_3$ in soluble fractions $F_1 + F_2$ .....	8.30	8.41
The percentage soluble fractions in whole samples.....	45.9	45.9
Therefore percentage of $\text{NO}_3$ calculated on whole sample..	3.81	3.86

Soil samples were taken at the same time that the green plants were gathered. The nitrates were estimated by the colorimetric method with phenoldisulphonic acid. The moisture in percentage and nitrates in parts per million are shown in fig. 1.

On June 20 the samples from the manure pile and from the knoll were taken for comparison. The nitrate content of the soil and that in the plant were as follows:

	Knoll sample	Manure pile sample
$\text{NO}_3$ in ppm in soil.....	300	29
$\text{NO}_3$ in percentage in plant (stem only).....	1.71	1.45

The high  $\text{NO}_3$  content in the soil of the knoll sample is probably due to the fact that some one, perhaps the gardener, had disturbed the soil by dragging his cultivator over it accidentally in cultivating his plot near by. The second reason is the better drainage and aëration in the knoll, and therefore better conditions for nitrification; but the striking fact is that high nitrate content in the soil did not bring about a proportional high nitrate content in the green plant organs. The rate of absorption increases with the aging of the plant; when the plants were about 25 days old, the nitrate in the stem was only 1.71 per cent. Eighteen days later (July 8) the nitrate content had risen to 8.58 per cent. During the same period branches grew from 0.1 to 14 inches, and their nitrate content rose to 12.50 per cent. This rapid increase in absorption of nitrates may partially be explained by the increase in extent of the absorbing roots from a radius of a few inches to about 2 ft.

The nitrate content in the roots, stems, and leaves is given in table II and is also shown in fig. 1. The nitrate content of the roots falls gradually from 1.85 per cent on June 3 to zero on June 20. At the same time nitrates in the leaves fall from 1.38 per cent to zero, while in the stem there is a gradual increase. There must be a definite reason for such differences. The differences may be due

to many causes. In the leaves protein synthesis is going on continuously in the presence of soluble carbohydrates. There is also synthesis of other organic nitrogen compounds, such as chlorophyll,

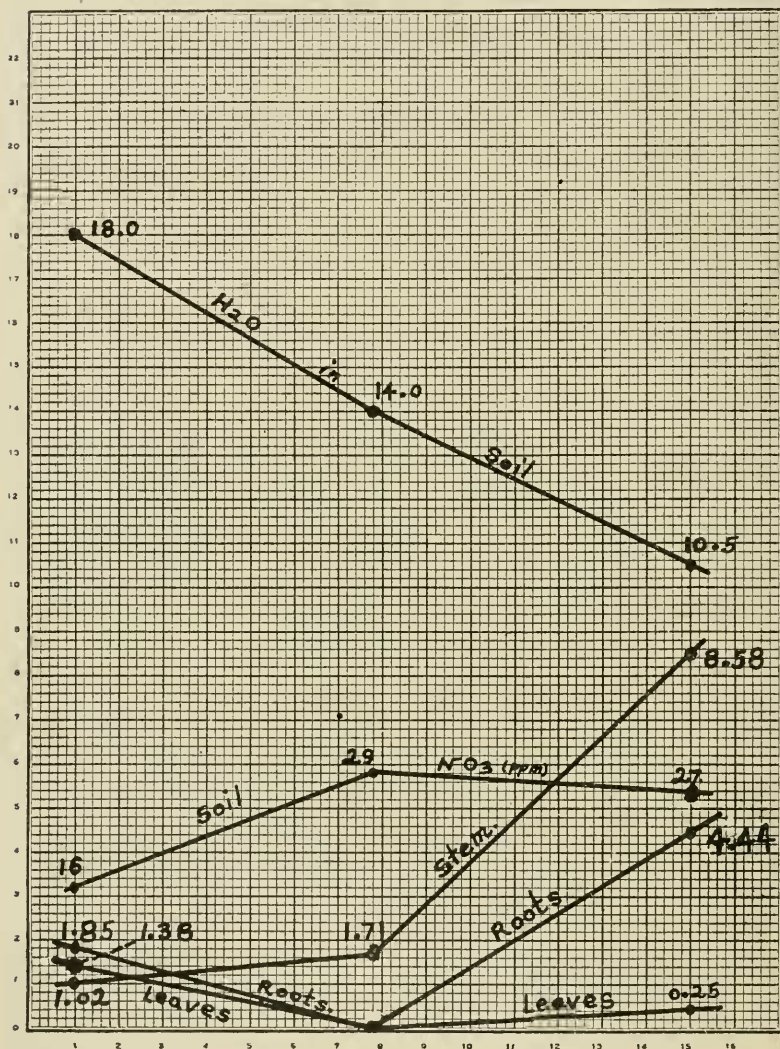


FIG. 1.—Relation of soil nitrification and nitrate intake by green plant organs; nitrate in soil expressed in parts per million; all other data calculated as percentage on dry weight basis.

phospholipins, etc., and all of the nitrates of the leaves seem to be used up in these syntheses. The nitrates are carried from the roots to other parts of the plants as fast as they are taken up from the soil. There may be as high a concentration of nitrates in the roots as in the soil (29 parts per million). This low concentration cannot be estimated by this method and would therefore be missed.

The stem and branches are the primary nitrate storage organs. The nitrate content rises as high as 8.58 per cent in the stem and 12.5 per cent in the branches during the early seed formation period. This high content is shown still more clearly by the ratio of nitrate nitrogen to the total nitrogen. This is 32.8 per cent for the roots, 51.85 per cent for the stems, 56.4 per cent for the branches, but only 1.25 per cent in the leaves. Curves showing this ratio in these organs at different stages are given in fig. 2. This large supply of nitrates in the stem and branches may be drawn upon heavily for further growth and seed production, although the supply seems more than adequate for these uses. There is also no reason for thinking that nitrate absorption ceases at this time. The extent to which this storage of nitrate is drawn upon by later development could be ascertained by the analysis of a set of samples taken late in the fall when seed formation and growth were complete. It is to be regretted that circumstances made such an analysis impossible for this paper.

It is worthy of note that the nitrate storage organs are the ones that made the most rapid growth in length, weight, and volume. The stem which rose from 8 inches on June 20 to 20 inches on July 8 at the same time increased in nitrate content from 1.71 to 8.58 per cent on dry weight basis. In addition to the stem there are numerous side branches which elongated from 0.1 to 14 inches in 18 days, making nearly 1 inch in 24 hours. At the same time there was an increase in percentage of nitrates per gram of dry matter from 1.71 on June 20 to 12.50 per cent on July 8. The rate of nitrate intake per gram per hour seems to follow a geometrical progression in each individual plant.

It appears that *Amaranthus* may be a very considerable factor in depleting soils of their nitrates. Also in case the weeds are burned the nitrogen stored is permanently lost from the soil. It

is a point of interest to know how generally this great power of absorbing and storing nitrates is possessed by weeds.

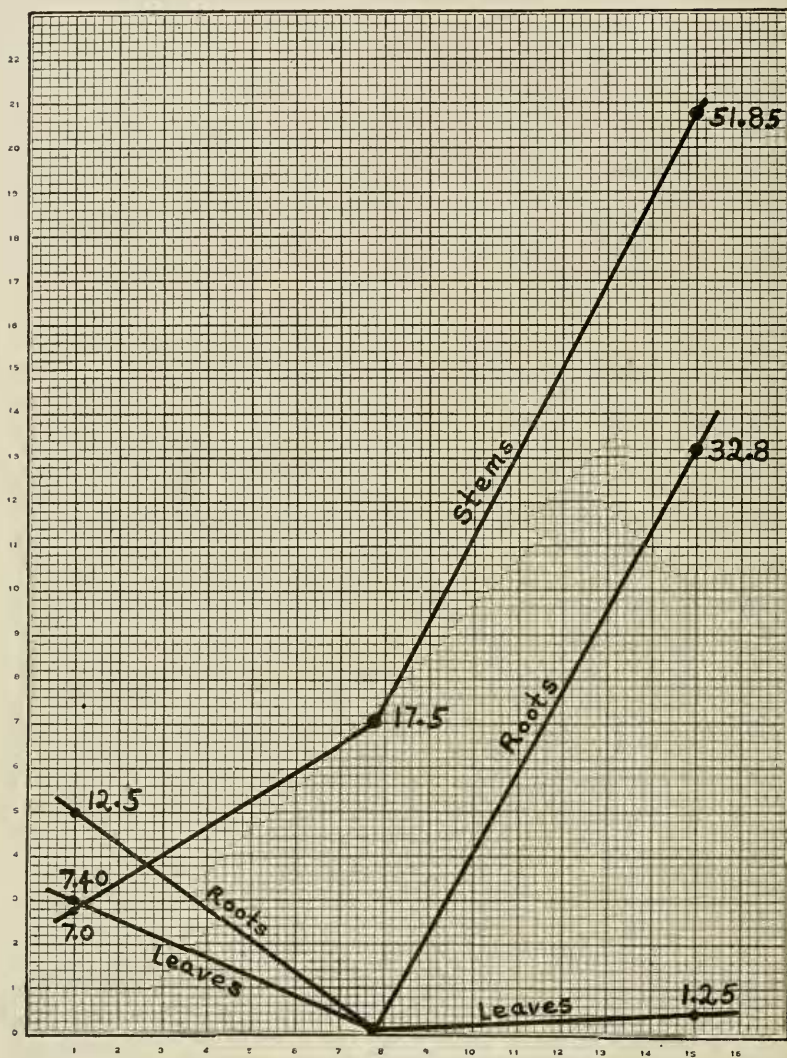


FIG. 2.—Ratio of nitrate nitrogen to total nitrogen; ratio for branches (not shown in figure) 56.4.

AMINO N.—The amino nitrogen was determined by the Van Slyke apparatus. The amino acids thus determined are chiefly

of the mono-amino-monocarboxylic acids. In each estimation only 2 cc. of the solution was used. The amino acid nitrogen deter-

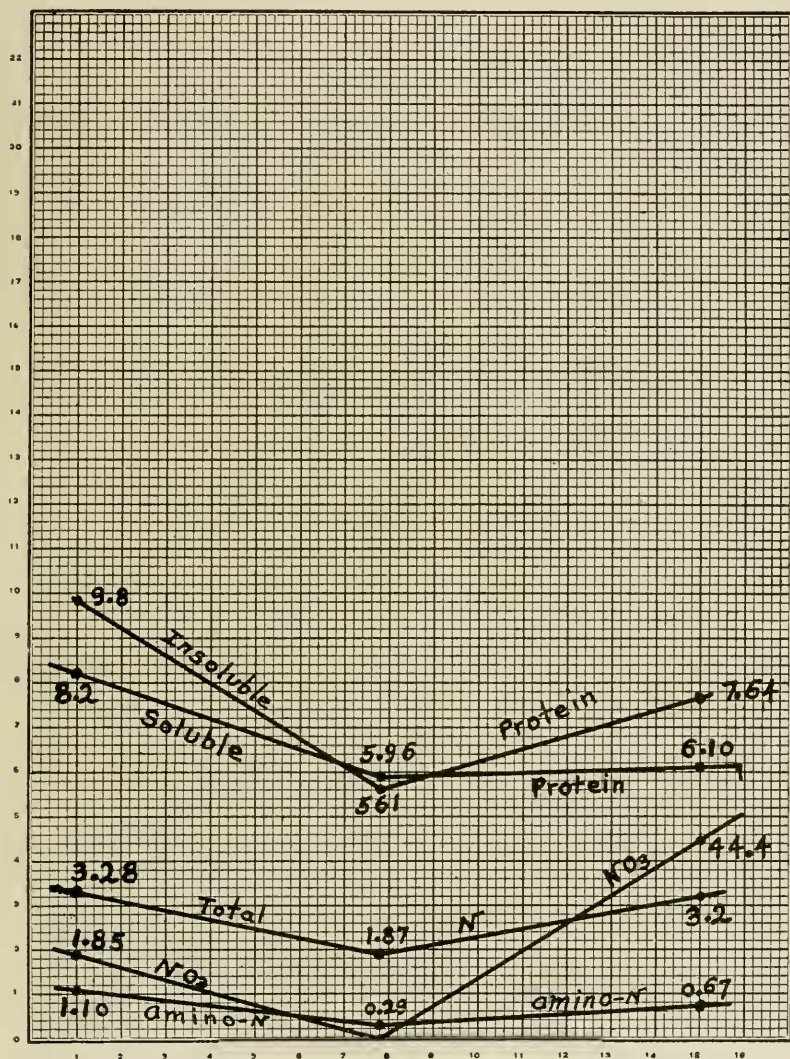


FIG. 3.—Nitrogen compounds in roots

mined throughout the season is given in table II. Curves showing the variation in roots, stems, and leaves are given in figs. 3, 4, and 5 respectively. In all the three organs the variation is very small.

In general the amino N varies directly with the insoluble protein; with a high protein content, high amino N; and low protein content, low amino N.

TABLE II  
THE NITROGEN COMPOUNDS IN THE GREEN PLANT

Material	Roots		Stems		Branches		Leaves	
	June 3 collection (1-4 inches)							
Total N.....	3.33	3.33	3.19	3.21	....	....	4.25	4.27
Nitrates NO <sub>3</sub> .....	1.77	1.93	0.99	1.04	....	....	1.36	1.40
Nitrate N.....	0.40	0.43	0.22	0.23	....	....	0.31	0.32
Amino N.....	1.09	1.13	0.43	0.43	....	....	0.97	0.96
Insoluble N.....	1.62	1.52	1.74	1.73	....	....	3.21	3.23
Insoluble protein...	10.18	10.25	10.92	10.80	....	....	20.20	20.30
Soluble protein....	8.24	8.17	7.67	7.92	....	....	4.58	4.52
June 20 collection (6-8 inches)								
Total N.....	1.85	1.88	2.39	2.21	....	....	4.56	4.51
Nitrates NO <sub>3</sub> .....	None	None	1.67	1.75	....	....	None	None
Nitrate N.....	None	None	0.38	0.40	....	....	None	None
Amino N.....	0.29	0.29	0.19	0.19	....	....	1.38	1.37
Insoluble N.....	0.86	0.91	0.95	0.97	....	....	3.66	3.59
Insoluble protein...	5.40	5.72	5.86	6.10	....	....	23.00	22.6
Soluble protein....	5.92	6.10	5.28	5.41	....	....	5.66	5.78
July 8 collection (20 inches)								
Total N.....	3.21	3.12	3.74	3.80	5.04	4.94	4.85	4.80
Nitrates NO <sub>3</sub> .....	4.41	4.47	8.40	8.75	12.50	12.40	0.25	0.246
Nitrate N.....	0.99	1.02	1.90	1.98	2.85	2.80	0.06	0.06
Amino N.....	0.63	0.70	0.35	0.34	0.48	0.49	1.44	1.42
Insoluble N.....	1.29	1.14	0.95	0.91	1.01	0.98	3.29	3.21
Insoluble protein...	8.10	7.17	5.96	5.71	6.35	6.15	20.62	20.08
Soluble protein....	6.16	6.05	5.60	5.70	7.42	7.30	9.42	9.62

INSOLUBLE PROTEIN.—The insoluble protein was calculated from nitrogen of the insoluble fraction (F<sub>3</sub>). The insoluble protein is given in table II, and curves showing fluctuation during the growing season are given in figs. 3-5. The insoluble protein falls and then rises again at maturity in the root, while in the leaves the fluctuation is in the opposite direction (see curves). In the stem the decline is in the early stage from 10.89 per cent (June 3) to 6.03 per cent (June 20). From that time on the curve is almost a straight horizontal line; therefore the rate of synthesis of the insoluble protein must have been keeping pace with the growth of the stem,

because the fall at this time is only from 6.03 to 5.84 per cent (almost within experimental error).

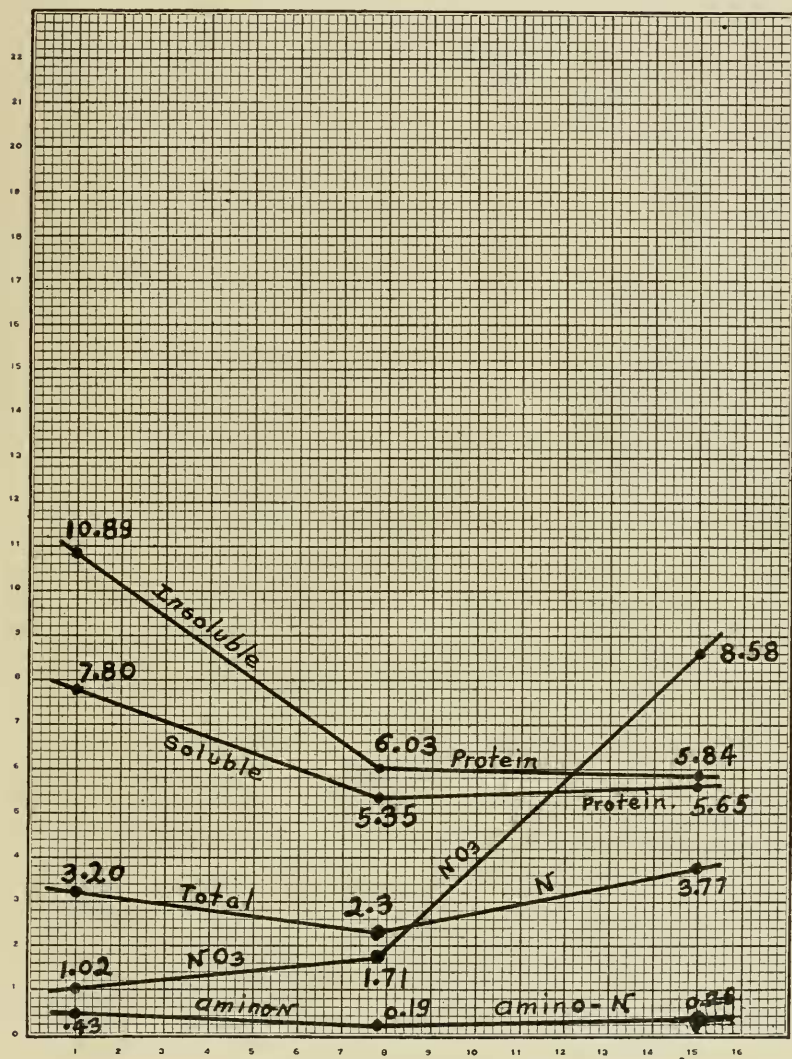


FIG. 4.—Nitrogen compounds in stems

**SOLUBLE PROTEIN.**—Soluble protein in  $F_1$  and  $F_2$  is computed from the Kjeldahl nitrogen determination. The nitrate nitrogen

was determined separately (by the method previously described); so the Kjeldahl determination was conducted without any modifica-

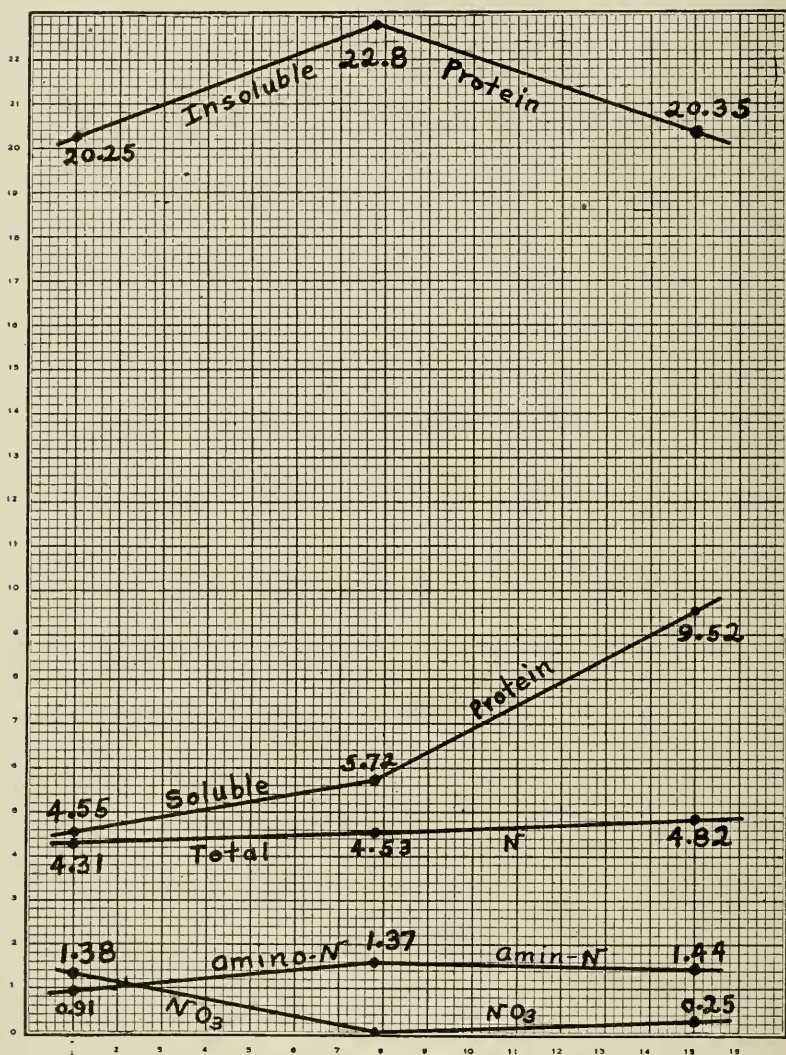


FIG. 5.—Nitrogen compounds in leaves

tion for nitrates. (Previously zinc was used, but this did not reduce any nitrate. Experiment with a known solution 0.5 per cent KNO<sub>3</sub>,

per official method by the use of salicylic acid and sodium thio-sulphate, reduced only 60 per cent of the nitrate into ammonia.)

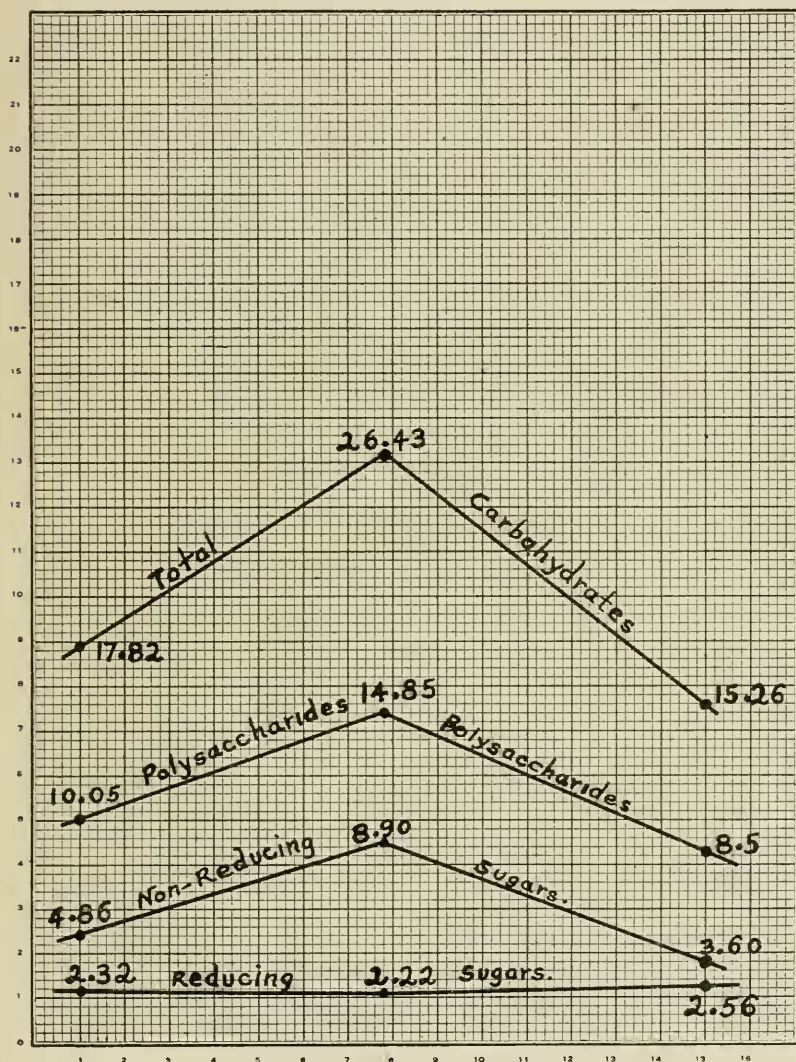


FIG. 6.—Different carbohydrates in roots

It is incorrect to call all this nitrogen as derived from protein, because part of the soluble nitrogen was from the breaking down

of the chlorophyll; but for comparison it is not out of place to calculate the N by the factor 6.29 to convert it into soluble protein

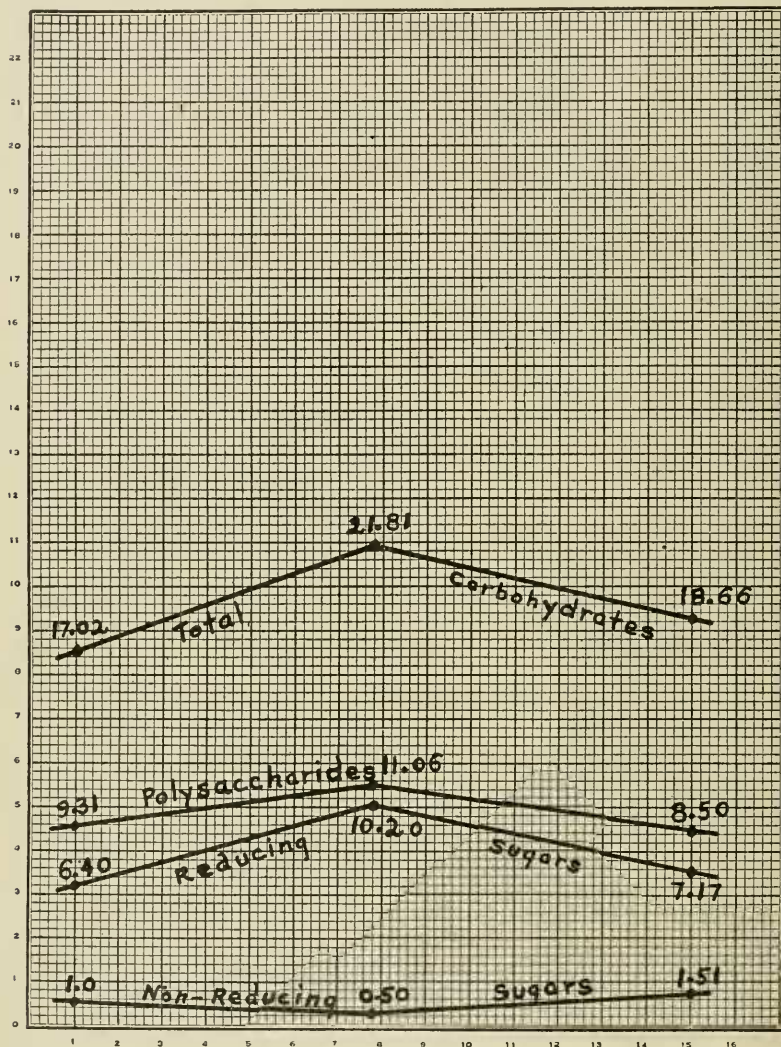


FIG. 7.—Different carbohydrates in stems

for temporary convenience in interpreting the results. The curves of the soluble protein in figs. 3-5 are self-explanatory, showing the variation throughout the season.

CARBOHYDRATES.—The carbohydrates were determined by the reduction method with Fehling solutions. The cuprous oxide

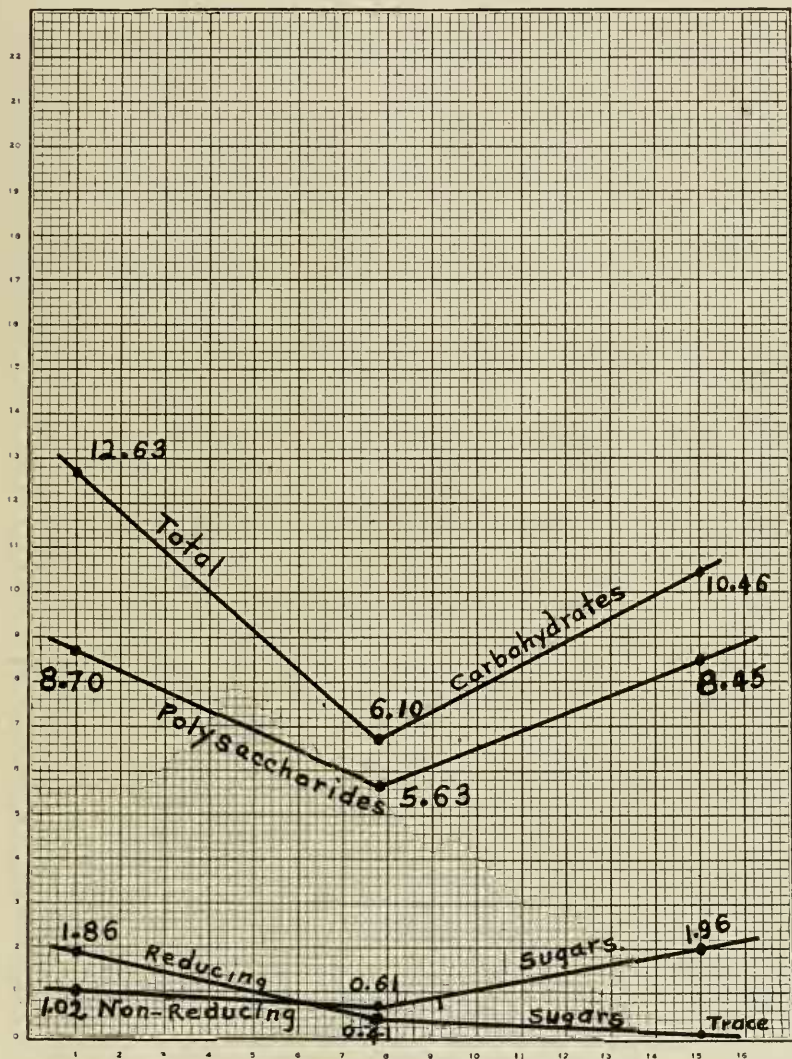


FIG. 8.—Different carbohydrates in leaves

obtained was dissolved in excess of ferric ammonium sulphate with  $\text{H}_2\text{SO}_4$  previously added. The ferrous ions produced by the oxidation of cuprous oxide were titrated against a  $\text{N}/20 \text{ KMnO}_4$

solution, in which 1 cc. represents 3.1 mg. of copper. The corresponding equivalents of the different sugars expressed in milligrams were found in the Munson-Walker table. The weight of sugar found divided by the material used gives the amount of sugar contained in 1 gm. of material.

The soluble carbohydrates are in  $F_1$  and  $F_2$ . The reducing sugar was first determined. The non-reducing sugar was obtained by subtracting the reducing sugar from the total sugar by hydrochloric acid hydrolysis at 67–69° C. for 10 minutes.

The insoluble carbohydrates are in  $F_3$ . They consist essentially of colloidal polysaccharides, the greater part of which was starch. The polysaccharides were determined by the Fehling solution after acid hydrolysis for 2.5 hours with a reflex condenser.

TABLE III  
THE CARBOHYDRATES IN THE GREEN PLANT

Material	Roots		Stems		Branches		Leaves		
	June 3 collection (1-4 inches)								
Total carbohydrates . . .	17.81	17.83	16.95	17.09	....	....	12.76	12.57	
Reducing carbohydrates	2.38	2.27	6.51	6.30	....	....	1.86	1.87	
Non-reducing . . . . .	5.43	5.46	1.16	1.44	....	....	2.14	2.05	
Polysaccharides . . . . .	10.00	10.10	9.28	9.35	....	....	8.76	8.65	
	June 20 collection (6-8 inches)								
	Total carbohydrates . . .	26.29	26.57	21.78	21.84	....	....	6.78	6.61
	Reducing carbohydrates	2.24	2.19	10.25	10.15	....	....	0.44	0.38
	Non-reducing . . . . .	9.30	9.43	0.53	0.57	....	....	0.63	0.67
	Polysaccharides . . . . .	14.75	14.95	11.00	11.12	....	....	5.71	5.56
	July 8 collection (20 inches)								
	Total carbohydrates . . .	15.32	15.21	18.69	18.63	15.18	15.07	10.51	10.41
	Reducing carbohydrates	2.85	2.54	7.15	7.20	5.00	5.10	Trace	Trace
	Non-reducing . . . . .	3.82	3.71	1.67	1.50	1.18	1.02	1.96	2.01
	Polysaccharides . . . . .	8.92	8.96	9.87	9.93	9.00	8.95	8.55	8.41

The percentage of the different carbohydrates of various organs estimated at different times throughout the growth period is tabulated in table III. Curves showing the changes in different sugar content in these organs are given in figs. 6-8. These curves show that in the roots the reducing sugars remain constant, while the

non-reducing sugars fluctuate throughout the season. This is just the reverse of what is found in the stems. In the leaves the

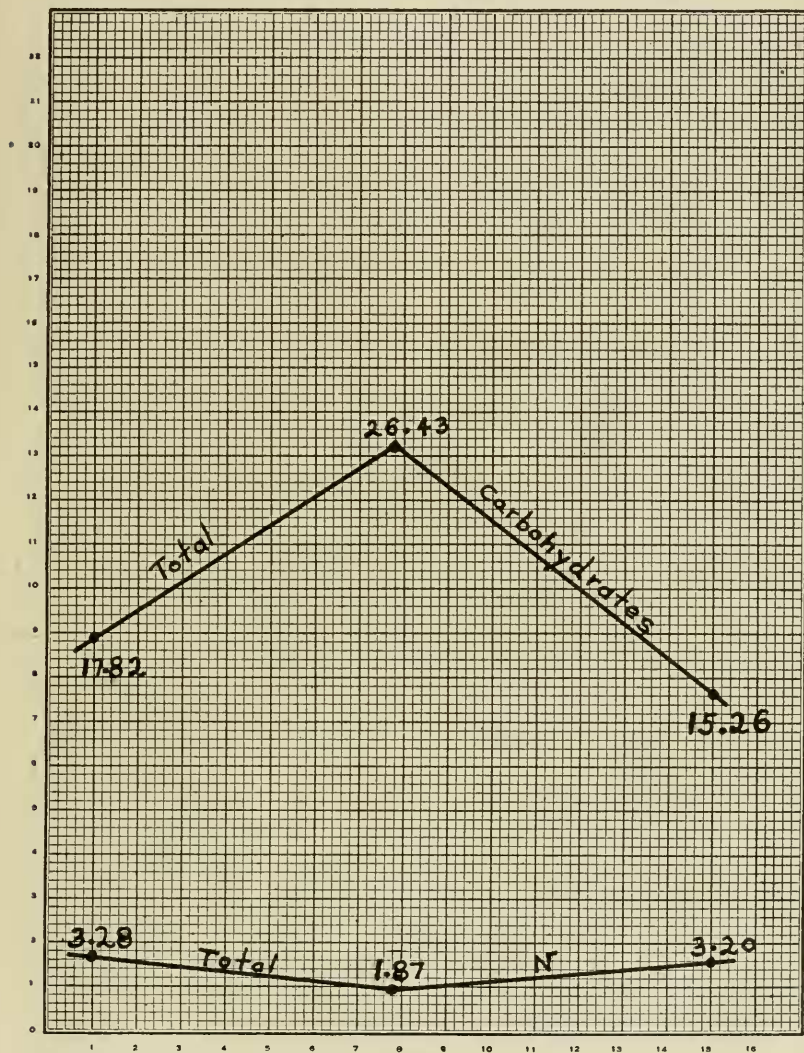


FIG. 9.—Reciprocal fluctuation of carbohydrate and nitrogen in roots (cf. figs. 3 and 6).

fluctuation of the reducing and non-reducing sugars is in the opposite direction; when the reducing sugars are high, the non-reducing

sugars are low, and the reducing type falls to zero at the time of seed formation.

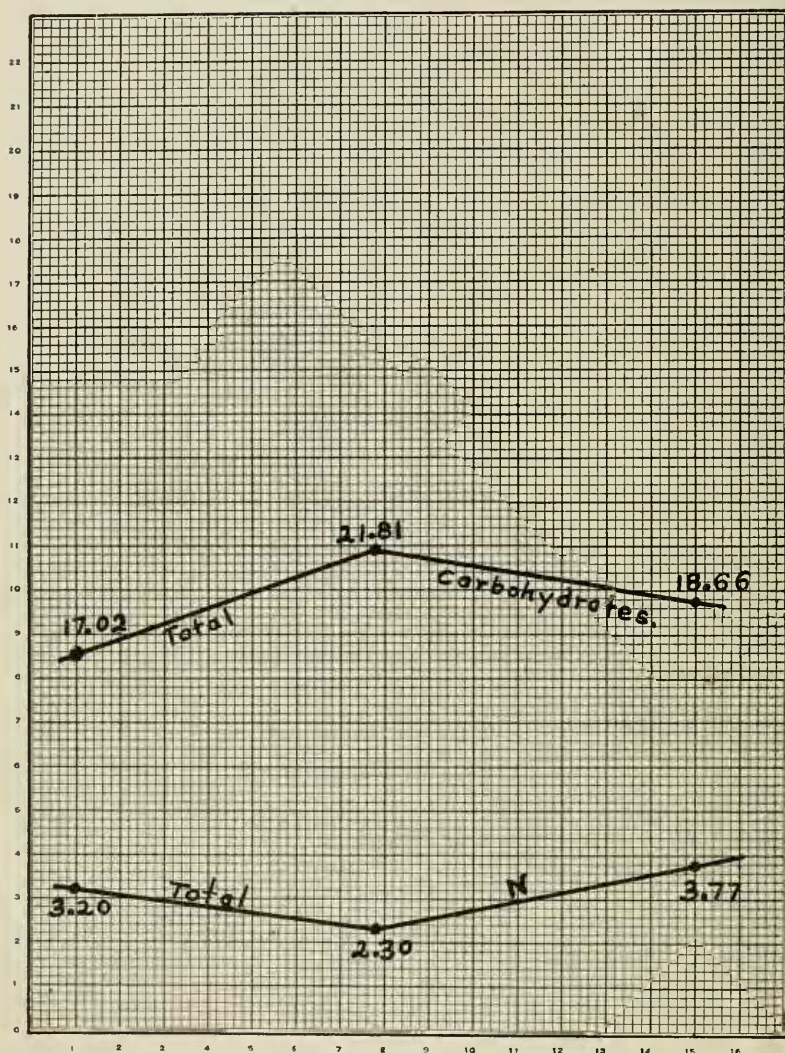


FIG. 10.—Reciprocal fluctuation of carbohydrate and nitrogen in stems (cf. figs. 4 and 7).

CARBOHYDRATE-NITROGEN RATIO.—According to the work of KRAUS and KRAYBILL on the tomato, high nitrogen in a plant is

accompanied by low carbohydrate. "Whatever the conditions under which a plant has been grown, considering the whole plant

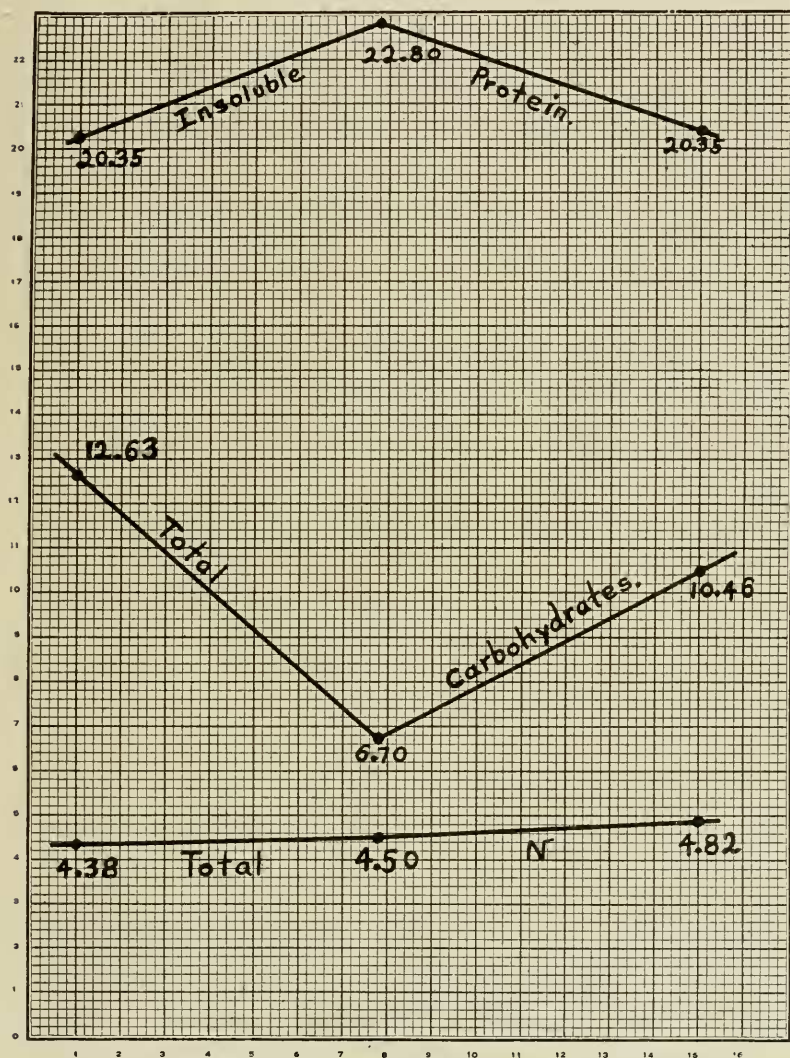


FIG. 11.—Reciprocal fluctuation of carbohydrate and nitrogen in leaves (cf. figs. 5 and 8); notice that carbohydrate reciprocates with insoluble protein and not with total nitrogen.

as a unit, increased total nitrogen and more particularly increased nitrate nitrogen are associated with increased moisture and

decreased free-reducing substances, sucrose, polysaccharides, and total dry matter."

In the work with *Amaranthus* plants I have found a similar situation so far as the relation between nitrogen and carbohydrate is concerned; that is, low nitrogen is accompanied by high carbohydrate and high nitrogen by low carbohydrate. Upon computing the reciprocal condition in the different fractions I find that the product of carbohydrate by nitrogen is not a mathematical constant, but that it varies considerably, sometimes decreasing as the development of the plant progresses. The product varies least in the stem and roots.

Let  $C_1$ ,  $C_2$ , and  $C_3$  be the carbohydrates and  $N_1$ ,  $N_2$ , and  $N_3$  denote the nitrogen, the sub-numbers representing the time of collection. If the carbohydrate and nitrogen hold a reciprocal relation, then  $\frac{C_1}{C_2} = \frac{N_2}{N_1}$ ,  $\frac{C_2}{C_3} = \frac{N_3}{N_2}$ , and  $\frac{C_3}{C_1} = \frac{N_1}{N_3}$ ; by clearing the fractions,  $C_1 \times N_1 = C_2 \times N_2 = C_3 \times N_3$ , etc., or carbohydrate  $\times$  nitrogen = constant K. Applying this principle, the following constants are obtained.

*Insoluble fraction ( $F_3$ )*

	JUNE 3	JUNE 20	JULY 8	Av. K.
Roots.....	15.7	13.06	10.92	13.23
Stems.....	16.20	10.62	9.20	12.01
Leaves.....	28.00	20.4	27.6	25.30

*Soluble fractions ( $F_1 + F_2$ )*

Roots.....	10.10	11.00	6.27	9.12
Stems.....	9.45	8.97	7.88	8.43
Leaves.....	2.39	2.97	3.15	2.84

These data show that the carbohydrate-nitrogen ratio is not a constant as we think of a constant in mathematics or physics. In plants where great fluctuation occurs in their substratum throughout different parts of the day and different times in the season, this disparity is no positive evidence that such a ratio does not exist. Secondly, regardless of the exactness of the ratio, this much is true, when the carbohydrates are high the nitrogen compounds are relatively low, and vice versa. Figs. 9-11 show this reciprocal

condition in the roots, stems, and leaves in the various stages of development.

#### SEEDS

A tissue analysis of the seeds was made in order to discover what important compounds were present and the distribution of these compounds in the various fractions. This gives one a more comprehensive knowledge of the chemical constitution of the plant.

PREPARATION.—The seeds were freed from chaff and cleaned in a breeze until all red seeds were removed and only plump black ones remained. These uniform seeds were then ground in a mortar with the pestle by taking a few at a time. A known quantity (25 gm.) was weighed out in triplicate for alcoholic digestion, and the tissues were fractionated and the carbohydrates, the nitrogen compounds, and the phosphorus determined.

TABLE IV  
SEEDS; SUMMARY OF TOTAL CONSTITUENTS

Material	A. blitoides		A. retroflexus		
H <sub>2</sub> O Av. 3 .....		9.45		8.61	
Total N .....	2.55	2.42	2.54	2.47	2.37
Total P .....	4.01	3.93	4.63	4.60	4.65
Total carbohydrates .....	48.27	48.43	47.22	47.03	47.37
Lipins .....	4.58	4.44	7.67	7.86	7.78
Ash (ignition) .....	3.50	3.68	4.27	4.19	4.14

DIFFERENT FORMS OF PHOSPHORUS.—After separating the seeds into fractions F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub>, the different phosphorus compounds were estimated in the three fractions. In each case the phosphorus was determined by the Pemberton-Kilgore method (10). The analysis of the different forms of phosphorus is given in table V, and the percentage ratio of these different forms to the total phosphorus is given in table VI. The inorganic phosphorus was estimated by the CHAPIN-POWICK method (4). The amounts obtained from fractions two and three (F<sub>2</sub>+F<sub>3</sub>) were combined and calculated as total inorganic phosphorus. The soluble organic phosphorus was obtained by subtracting the inorganic phosphorus of fraction two only from total phosphorus in the same fraction (F<sub>2</sub>). The

phosphoprotein phosphorus was split off from the insoluble fraction ( $F_3$ ) by digesting a weighed amount (1-3 gm.) with 1 per cent NaOH solution at 37-40° C. for 48 hours in an incubator, after which the solution was neutralized with acetic acid and made up to volume. The insoluble material was separated from the filtrate by the use of the centrifuge. The filtrate obtained was tested for phosphorus with magnesia mixture. The magnesium ammonium phosphate precipitated out by standing overnight was dissolved, and the phosphorus was reprecipitated as ammonium phosphomolybdate and then titrated against a standard alkali (8). The nucleoprotein phosphorus was estimated by the difference between the total phosphorus minus the phosphoprotein and the inorganic phosphorus in the two fractions ( $F_2 + F_3$ ). The lipin phosphorus was determined by taking an aliquot of the ether soluble fraction ( $F_1$ ). The ether was first driven off on a steam bath before acid digestion and the percentage of this phosphorus was estimated in the same way.

TABLE V  
DIFFERENT FORMS OF PHOSPHORUS (PERCENTAGE P) IN SEEDS

Material	A. blitoides		A. retroflexus		
Inorganic P.....	0.133	0.137	0.131	0.123	0.132
Lipin P.....	0.014	0.013	0.019	0.020	0.017
Soluble organic P.....	0.011	0.012	0.023	0.033	0.034
Phosphoprotein P.....	1.240	1.340	1.840	1.950	1.700
Nucleoprotein P.....	2.610	2.430	2.620	2.470	2.670
Total P.....	4.008	3.932	4.633	4.596	4.649

TABLE VI  
RATIO OF DIFFERENT P TO TOTAL P (PERCENTAGE P) IN SEEDS

Material	A. blitoides		A. retroflexus		
Inorganic P.....	3.32	3.49	2.83	2.65	2.84
Lipin P.....	0.35	0.33	0.41	0.44	0.37
Soluble organic P.....	0.28	0.31	0.49	0.72	0.73
Phosphoprotein P.....	31.00	34.10	39.74	42.40	38.50
Nucleoprotein P.....	65.30	61.80	50.60	53.70	57.50
Total P.....	100.25	100.00	100.07	99.91	99.94

Tables V and VI show the percentage of the different forms of phosphorus. The data in these tables show that about 96 per cent

of the total phosphorus is in the organic combination in the seed, existing (perhaps) as phosphoprotein and nucleoprotein phosphorus. Both of these forms are insoluble in water, alcohol, ether, and alcohol-water solvents. The inorganic phosphorus is relatively low, and the writer believes that the figures given for inorganic phosphorus in table V are even too high, because the greater part of the inorganic phosphorus was obtained from the insoluble fraction  $F_3$  (4). Moreover, there is no proof that the reagents used did not break down some of the organic phosphorus. The lipin phosphorus is very low, varying from 0.014 per cent in *A. blitoides* to 0.019 in *A. retroflexus*, calculated on dry weight basis. It is interesting to know that in all cases the different forms of phosphorus are relatively higher in *A. retroflexus* than the corresponding forms in *A. blitoides*.

TABLE VII

DIFFERENT NITROGEN COMPOUNDS IN SEEDS (PERCENTAGE DRY WEIGHT)

Material	A. blitoides		A. retroflexus		
Total N.....	2.550	2.420	2.540	2.470	2.370
Nitrates $\text{NO}_3$ .....	0.200	0.212	0.194	0.193	0.205
Amino N.....	0.096	0.095	0.089	0.090	0.090
Lipin N.....	0.027	0.027	0.031	0.032	0.033
Soluble proteins.....	2.260	2.270	2.660	2.790	2.890
Insoluble proteins.....	12.640	12.450	12.700	12.820	12.250
Total proteins.....	14.900	14.720	15.360	15.610	14.140

TABLE VIII

RATIO OF DIFFERENT N TO TOTAL N (PERCENTAGE DRY WEIGHT)

Material	A. blitoides		A. retroflexus		
Total insoluble N.....	83.20	82.00	80.40	79.00	77.00
Total soluble N.....	16.97	17.93	19.62	21.01	23.23
Total.....	100.17	99.93	100.02	100.07	100.23
Nitrate N.....	1.77	1.88	1.73	1.77	1.97
Lipin N.....	1.06	1.13	1.24	1.29	1.41
Amino N.....	4.68	4.65	3.50	3.66	3.84
Other soluble organic N.....	12.29	13.28	16.12	17.41	19.37

NITROGEN COMPOUNDS.—The distribution of nitrogen in the different fractions of the seeds is about the same as that of phosphorus. The insoluble nitrogen comprised 80–83 per cent of the total nitrogen (tables VII and VIII). The soluble fractions

contain only 17-20 per cent of the total, and most of it exists as organic nitrogen. The portion representing inorganic nitrogen is the nitrate nitrogen, which is relatively small. Calculated as nitrates ( $\text{NO}_3$ ), the seeds contain 0.20 per cent, and this is equivalent to 1.80 per cent of the total nitrogen (tables VII and VIII). The lipin nitrogen is very small, only 0.027 per cent in *A. blitoides* and 0.032 per cent in *A. retroflexus*. These represent 1.10 and 1.31 per cent respectively of the total nitrogen content in these two seeds. In general a high percentage of insoluble phosphorus is accompanied by a high percentage of insoluble nitrogen, and a low percentage of soluble phosphorus by a low percentage of soluble nitrogen.

CARBOHYDRATES.—The polysaccharides are the predominating sugars in these seeds. *A. retroflexus* seeds contain 46 per cent and *A. blitoides* 47.75 per cent polysaccharides (on dry weight

TABLE IX

CARBOHYDRATES IN SEEDS (PERCENTAGE DRY WEIGHT)

Material	A. blitoides		A. retroflexus		
	None	None	None	None	None
Lipin sugars.....	None	None	None	None	None
Reducing sugars.....	None	None	None	None	None
Non-reducing sugars.....	0.67	0.68	1.12	1.13	1.17
Polysaccharides.....	47.60	47.75	46.10	45.90	46.20
Total.....	48.27	48.43	47.22	47.03	47.37
Ratio of different sugars to the total carbohydrates					
Non-reducing.....	1.38	1.40	2.37	2.40	2.47
Polysaccharides.....	98.65	98.60	97.60	97.60	97.60
Total.....	100.03	100.00	99.97	100.00	100.07

basis). If these sugars are calculated on the dry basis of the total sugars, the polysaccharides represent 97.60 and 98.60 per cent respectively in these two species. A striking contrast is seen on comparing the amount of polysaccharides in the green plant organs (figs. 6-8), which vary only slightly throughout the growing period, with that found in the seeds. In the growing period the highest percentage of polysaccharides was only 14.85, while that of the seeds was 47. In addition to this noticeable contrast, the soluble

sugars, both reducing and non-reducing, were comparatively high in the green plant organs (6-8 per cent), while those of the seeds are low (0.67-1.14 per cent).

**LIPIN FRACTION.**—The percentage of this fraction is 4.5 for *A. blitoides* and 7.78 for *A. retroflexus*. A closer examination of this fatlike substance shows that it contains phosphorus and nitrogen. The percentage of this nitrogen and phosphorus is very low (calculated on dry weight basis of whole sample). The presence of nitrogen and phosphorus indicates that the seeds contain phosphotides. The atomic ratio of nitrogen to phosphorus was determined by dividing the percentage by their respective atomic weights. The atomic ratio of N:P for *A. blitoides* is 1:2.3, and that for *A. retroflexus* is 1:2.6. This shows that other forms of phosphorus must be present than that existing in "ideal lecithin," which implies that the atomic ratio of N:P = 1:1 (5, 13).

In addition to lecithin, a phytosterol was present in the seeds. This could not be quantitatively determined by using animal cholesterol as a standard, because animal cholesterol (17) has a different tint from that of the plant. BUCHARD's color reaction gives a deep blue color for animal cholesterol, while for the seeds it gives a yellowish green. The amount of phytosterol in these two species of seeds was compared. Assuming that the phytosterol in *A. blitoides* is unity, that in *A. retroflexus* is 2.8.

TABLE X

CONSTITUENTS OF LIPIN FRACTION ( $F_1$ ) OF SEEDS (PERCENTAGE DRY WEIGHT)

Material	A. blitoides			A. retroflexus		
Lipin, fats, etc. (wet basis)	4.15	4.07	4.23	7.02	7.18	7.09
Lipin (dry basis).....	4.58	4.44	4.66	7.67	7.86	7.78
P in $F_1$ .....	0.300	0.300	.....	0.240	0.260	0.220
P calculated in whole sample	0.014	0.014	.....	0.019	0.020	0.017
N in $F_1$ .....	0.600	0.640	.....	0.402	0.400	0.420
N calculated in whole sample	0.027	0.027	.....	0.031	0.031	0.033

**INORGANIC ELEMENTS.**—The 1917 seeds of *Amaranthus retroflexus* were used for the estimation of the inorganic elements. The percentage of total ash given in table IV is 3.59 per cent for *A. blitoides* and 4.20 for *A. retroflexus*, but this is actually too low

for the inorganic elements present in the seeds, because the total phosphorus alone is 4.0 and 4.60 per cent respectively for the two species. This discrepancy is due perhaps to the loss of nitrates and part of the sodium, potassium, etc., in burning in the electric muffle. In the following analysis of the inorganic elements acid digestion (concentrated  $\text{H}_2\text{SO}_4$  + concentrated  $\text{HNO}_3$ ) was used for the kations and alkaline fusion for the anions. The potassium was determined directly in the presence of all other elements except ammonia ( $\text{NH}_4$ -ion) and strong acid, as  $\text{K}_2\text{NaCO}(\text{NO}_2)_6 - \frac{1}{2} \text{H}_2\text{O}$  (11). Magnesium was estimated by the volumetric method as  $\text{NH}_4\text{MgAsO}_4$  (7). The percentage of inorganic elements is as follows:

INORGANIC SALTS (*A. retroflexus*); 1917 SEEDS

	Percentage	Percentage
Silica.....	0.42	0.40
$\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3$ .....	0.53	0.56
$\text{CaO}$ .....	0.54	0.53
$\text{MgO}$ .....	0.82	0.84
$\text{K}_2\text{O}$ .....	0.38	0.35
$\text{Na}_2\text{O}$ .....	no determination	
$\text{Cl}$ .....	Trace	Trace
$\text{SO}_3$ .....	0.34	0.33
$\text{P}_2\text{O}_5$ .....	8.90	8.85
$\text{N}_2\text{O}_3$ (nitrates).....	0.12	0.123
Total (not including $\text{Na}_2\text{O}$ ).....	12.05	11.98

### Discussion

In spite of the inaccuracy of BOUTIN's method for the determination of the nitrates, his results are probably not far from correct. He stated his results in percentage of  $\text{KNO}_3$  as shown in the second column of table XI. I have calculated these as percentage of  $\text{NO}_3$ , as shown in the third column. His percentages of nitrates are not far from those found by me for the stems (8.57 per cent) and branches (12.50 per cent) of *A. retroflexus*, July 8 collection, as shown in table II.

	Percentage $\text{KNO}$	Percentage equivalent in $\text{NO}$
<i>A. retroflexus</i> .....	22.77	13.92
<i>A. blitum</i> .....	11.68	7.17
<i>A. ruber</i> .....	16.0	9.82

Table XI shows the results of the analyses of the seeds by various authors. In the main there is fairly close agreement, but in some cases there are considerable discrepancies. The discrepancies can probably be explained by the different chemical methods used by the various authors and by the lack of uniformity in the different crops analyzed.

TABLE XI

COMPARISON OF SOME OF THE ANALYSES ON *Amaranthus* SEEDS

MATERIAL	PAMMEL AND DOX		HARDING AND EGGE			WOO	
	A. blitoides	A. retroflexus	A. retroflexus			A. blitoides	A. retroflexus
			20 mesh	72 mesh	Oven dry		
Condition of seeds.....			Non-	uniform		Matured uniform	Matured uniform
H <sub>2</sub> O.....				11.28	8.60	Av. of 3	Av. of 3
Lipins (fats).....	Little*	Little*		7.92	8.46	9.45	8.61
Polysaccharides.....	Abundant	Abundant	39.77	40.98	44.83	4.56	7.77
Reducing sugars.....			Trace	Trace	Trace	47.68	47.21
Non-reducing sugars (sugar after inversion).....						None	None
Nitrogen.....	1.88	2.49	2.08	2.15	2.35	0.67	1.14
Protein.....	11.75	15.59	18.57	10.13	20.93	2.48	2.46
Ash.....			4.33	4.46	4.88	14.81	15.03
						3.59	4.20

\* Microchemical test.

From the results of this study it would seem that *Amaranthus retroflexus*, and probably other species of the same genus, can bear, as they ordinarily do bear, large amounts of free nitrates without being forced out of reproduction into extreme vegetation. This genus apparently is endowed with a very high capacity for nitrate absorption, as well as for maintaining its full seed production power in the face of a great excess of free nitrates. In this respect it seems to differ from the tomato studied by KRAUS and KRAYBILL, and probably from many other plants. Considering all angiosperms, it is likely that, due to hereditary characters, there is a great range of ease with which plants can be forced to excessive vegetation by extreme nitrate supply within the plant. It is well known that a given level of fertility that will throw small grains into extreme straw production with deficiency of grain will give excellent grain production in corn. This may be due to the lower nitrate absorbing power of the corn, to its greater

photosynthetic activity to balance the nitrates absorbed, or to the higher carbohydrate-nitrogen ratio accompanying best grain production. Which of these three possibilities really determines the situation can only be answered by such studies as those made or suggested on the tomato by KRAUS and KRAYBILL, or studies of the type made in this paper. It is evident, however, that there is need of numerous studies of the carbohydrate-nitrogen ratio in plants, both in regard to the factors affecting this ratio and the effect of the ratio on plant characters. As was suggested in the review of the literature at the beginning of this article, such studies are likely to throw much light on other physiological features than vegetation and reproduction.

### Summary

1. There is a large amount of nitrate in the organs of *A. retroflexus*. The stem and branches are the primary nitrate storage organs. The rate of nitrate absorption increases with the aging of the plant, perhaps partly being due to the development of the root system with numerous branching rootlets, increasing the radius of the feeding area from a few inches to 2 ft. or more.

2. This high capacity for nitrate absorption and storage must be an important factor in making *Amaranthus* a very successful competitor against cultivated plants, so effectively withdrawing as it does the nutrient element most commonly limiting plant production. It would be interesting to know how generally and to what degree weeds possess this power.

3. The carbohydrates and nitrogen compounds fluctuate throughout the growing period. The fluctuation of the carbohydrates is in the reverse order of the nitrogen compounds. This inverse ratio is not a truly mathematical constant, but in general when the carbohydrates are high the nitrogen compounds are low, and vice versa. As the nitrate nitrogen composes more than 50 per cent in the stems and branches, there is a possibility that nitrates have some modifying effects on this reciprocal relationship. This inverse ratio is due partly to the synthesis of protein, chlorophyll, phospholipin, and other organic nitrogen compounds at the expense of the soluble carbohydrates.

4. Tissue analysis of the seeds shows the distribution of different forms of phosphorus in the various fractions. The organic phosphorus, which consists chiefly of phosphoprotein and nucleoprotein phosphorus, is high, and that of the inorganic form is low.

5. The distribution of nitrogen in seeds is in the same order as that of the phosphorus. The insoluble portion contains 80-83 per cent of the total. The soluble part varies from 17 to 20 per cent, most of which is in the organic form. The inorganic form is represented by the nitrate nitrogen.

6. The predominating sugars in the seeds are the polysaccharides. These compose nearly one-half of the total dry weight of the seeds. In both *A. retroflexus* and *A. blitoides* there is absence of lipin sugars in  $F_1$  and reducing sugars in  $F_2$ . Only a small amount of non-reducing sugars was present in the two varieties.

7. The presence of nitrogen and phosphorus in the lipin fraction indicates that the seeds contain phosphatides. Phytosterol was also present. By comparison, *A. retroflexus* has 2.8 times as much as *A. blitoides*.

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